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Human Mutation

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# From Lowe syndrome to Dent disease: correlations between mutations of the OCRL1 gene and clinical and biochemical phenotypes

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## ABSTRACT

Mutations of OCRL1 are associated with both the oculocerebrorenal syndrome Lowe, a multi-systemic and Dent-2 disease, a renal tubulopathy. We have identified a mutation in 130 Lowe syndrome families and 6 affected by Dent-2 disease with 51 of these mutations being novel. No founding effect was evidenced for recurrent mutations. Two mutations initially reported as causing Dent-2 disease were identified in patients, including two brothers, presenting with Lowe syndrome thus extending the clinical variability of OCRL1 mutations.

mRNA levels, protein content and $PiP_2$-ase activities were analyzed in patient's fibroblasts. Although mRNA levels were normal in cells harbouring a missense mutation, the OCRL1 content was markedly lowered suggesting that enzymatic deficiency resulted mainly from protein degradation rather than a catalytic inactivation as usually reported. Analysis of a splicing mutation that led to the elimination of the initiation codon evidenced the presence of shortened forms of OCRL1 that might result from the use of alternative initiation codons. The specific mapping of the frameshift and nonsense mutations, exclusively identified in exons 1-7 and exons 8-23 respectively for Dent disease and Lowe syndrome together with the possible use of alternative initiation codons might be related to their clinical expression i.e. Lowe syndrome or Dent-2 disease.



**KEYWORDS :**

OCRL1 – Lowe syndrome – Dent 2 disease – Phosphatidylinositol 4,5 biphosphate

## INTRODUCTION

### Lowe syndrome

The oculocerebrorenal syndrome of Lowe (OCRL [MIM#309000]) is a rare X-linked multisystem disorder presenting with major abnormalities in the eyes, the kidneys and the central nervous system [Lowe et al., 1952; Loi, 2006]. OCRL is a rare disease with a prevalence estimated between 1 and 2 boys per million people. Ocular abnormalities include a constant prenatal development of cataracts and frequent associated signs such as glaucoma, microphthalmos, decreased visual acuity and corneal keloid formation. Neonatal hypotonia, intellectual impairment and areflexia are also cardinal features. The majority of patients have a cognitive delay and behavioural troubles including temper tantrums and aggressiveness are frequently noted. Brain magnetic resonance imaging (MRI) may show periventricular cystic lesions [Schneider et al., 2001; Loi, 2006]. Fanconi syndrome, a generalized impairment of the proximal tubular cells functions, is a major feature.

Onset of the tubular dysfunction can vary between patients and the severity tends to worsen with age. Low molecular weight proteinuria (LWMP) is invariably present and aminoaciduria, hypercalciuria and bicarbonaturia are frequently included. Progressive glomerular dysfunction leads usually to renal failure. Skeletal muscle abnormalities may develop as secondary consequences of hypotonia or renal dysfunction. Nontender joint swelling and subcutaneous nodules are also frequently described in affected patients and may reflect a primary abnormality of connective tissue growth. Lowe syndrome results from mutations of the *OCRL1* gene (MIM #300535) that encodes a phosphatidyl inositol 4,5 biphosphate ($PI(4,5)P_2$) phosphatase.

### Dent disease

Dent's disease (Dent-1, MIM#300009), is a X-linked proximal renal tubulopathy, characterized by LWMP, hypercalciuria and progressive renal insufficiency. In addition, other features of proximal tubular dysfunction such as glycosuria, aminoaciduria, phosphaturia, uricosuria or complete Fanconi syndrome may also be present [Dent and Friedman, 1964; Thakker, 2000]. Except for rickets noted in some patients; no extra-renal manifestations of the disease have been reported.

Mutations in the *CLCN5* gene (MIM #300008) encoding the renal voltage-gated chloride channel CLC-5 have been reported in 60% of patients with Dent's disease [Lloyd et al., 1996, Hoopes et al., 2004]. ClC-5 is mainly localized in subapical endosomes of epithelial kidney cells and contributes to the acidification of intra-endosomal compartments and participates in membrane recycling in the proximal tubule [Devuyst et al., 1999; Piwon et al., 2000]. A recent study showed that mutations in the *OCRL1* gene can lead to a Dent-like phenotype (Dent-2 disease, DD-2, MIM #300555) that can present as a clinical intermediate between Lowe syndrome and Dent disease [Hoopes et al., 2005; Bökenkamp et al., 2009]. Nevertheless, DD-2 patients harbouring an OCRL1 mutation displayed comparable decrease in PI(4,5)P2ase activity and OCRL1 content to those reported for patients affected by Lowe syndrome [Hoopes et al., 2005; this study].



**OCRL1**

OCRL1 is a type II inositol polyphosphate 5-phosphatase that participates into the Lowe, 2005]. OCRL1 has been originally localized to the Trans-Golgi Network and to lysosomes [Olivos-Glander et al., 1995; Dressman et al., 2000; Ungewickell et al., 2004] and more recently to endosomes [Choudury et al., 2005; Hyvola et al., 2006; Erdmann et al., 2007]. Abnormalities in the actin cytoskeleton have been demonstrated in Lowe fibroblasts

[Suchy and Nussbaum, 2002] and OCRL1 was recently proposed to play a role in regulating membrane trafficking and actin dynamics [Lowe, 2005; Faucherre et al, 2005].

The OCRL1 gene spans 52278 bp on chromosome Xq24.26 [ENSG00000122126] and contains 24 exons with exon 18a as an alternatively spliced exon mostly expressed in brain [Attree et al., 1992; Nussbaum et al., 1997]. The ATG start codon was initially assigned to exon 2 [Attree et al, 1992], however recent reports mapped the initiation codon in exon 1 [Hyvola et al., 2006; Erdmann et al, 2007; Mao et al., 2009]. Accordingly the 5156 bp cDNA (NM_000276) encodes a protein monomer of 901 amino-acids (MW = 103 226 kDa).

This paper reports 51 novel mutations of the *OCRL1* gene associated either with Lowe syndrome or Dent-2. Correlations between the mutations and their expression at the mRNA, functional and protein levels will be discussed in regard to their clinical expression. Based on these data, putative mechanisms to explain how mutations of the OCRL1 protein express as heterogeneous clinical entities will be discussed.

## MATERIAL AND METHODS

### Patients and samples

A panel of 175 non-related families addressed to the laboratory for suspicion of Lowe syndrome has been investigated. Among the male patients addressed for suspicion of Lowe syndrome, 124 index cases presented with full criteria of Lowe syndrome. 15 mothers of deceased children likely to be affected by Lowe syndrome were also investigated for mutations in the *OCRL1* gene. French clinical centres addressed 58% of the families with the remaining families originating from Europe (18%), Middle-East (5%), North-America (5%), Asia (6%) and Australia (3%).

41 patients affected by Dent's disease were additionally included in the study. All patients originated from France, they did not show cataract or noticed neurological troubles and did not harbour mutations in the *CLCN5* gene.

Blood samples and, whenever possible, fibroblasts, were obtained from the affected patients and/or from their relatives. An informed consent allowing genetic studies was obtained from all patients included in the study.

**Mutation detection**

Genomic DNA was isolated using standard procedures. The 24 exons of the *OCRL1* gene were amplified from genomic DNA as described previously [Monnier et al., 2000]. For mutational analysis, PCR-amplified DNA products were subjected to direct automated sequencing on an ABI 3130 DNA Analyser (Life Technologies, Carlsbad, CA, USA).

Alternatively RNA was extracted from 2-5 $10^6$ fibroblast cells using the Trizol® reagent (Life Technologies, Carlsbad, CA, USA). RNA was dissolved in 50 µl of RNAse-free water. Reverse transcription was performed by using 0.2 mg of total RNA at 48°c for 60 minutes with 50 U of Expand Reverse Transcriptase (Roche, Basel, Switzerland) and in the presence of oligo-dT and the following specific primer (5'-AACTTTGGCTTGGCAATATAAGTC). The resulting cDNA was then amplified and sequenced as previously described.[24] Mutations responsible for aberrant sized transcripts were characterized by direct sequencing of the corresponding exon and intron-exon junctions.

Mutation assignment was based on the cDNA sequence (Genbank # NM_000276) using the first coding ATG of exon 1 as initiation codon according to international guidelines for description of sequences variants (http://www.hgvs.org/mutnomen). This resulted in an in frame addition of 51 nucleotides at the 5' end of the cDNA and of 17 amino acids at the N terminal end of the protein in comparison with the initial nomenclature [Attree et al., 1992].

None of the novel variants identified in this study were found after screening of 200 chromosomes from the general population. Genomic rearrangements were evidenced using data obtained from PCR screening or MLPA analysis in probands. In families harbouring a genomic rearrangement, the carrier status of mothers was determined using a homemade MLPA® assay (MRC Holland, Amsterdam, The Netherlands) [Coutton et al, 2010].

**Quantitative transcript analysis**

Quantitative PCR was performed using 100 ng of cDNA in a final volume of 25 µl and specific primers for the *OCRL1* gene (F-5'-CGAGCTGTATCAGCGATGTC; R-5'-GGAGGCCTCAGGAGAAGACT) and the *GAPDH* gene used as control (F-5'-CATCAAGAAGGTGGTGAAGC; R-5'- GAGCTTGACAAAGTGGTCGT). The OCRL1 primers allowed the amplification of a 197 bp fragment spanning exon 21 to 23. Duplicated samples were PCR were run on a iQ Cycler apparatus in presence of the iQ SYBR Green Supermix containing 500 nM primers (Biorad, Hercules, CA, USA).

**Enzymatic assay for PI(4,5)$P_2$ phosphatase activity**

Activity was assayed as described previously on cell extracts prepared by freeze-thawing of fibroblasts obtained from affected and non affected patients except that each assay included 50 µg of fibroblast protein and that quantification was performed by direct analysis of the TLC plates using a Beta Imager 2000 (Biospace, Paris, France) [Suchy et al., 1995; Satre et al., 1999].

**Western blot analysis**

For western blot analysis, aliquots of cell extracts (25 µg of protein) were resolved by an 8% SDS-polyacrylamide gel electrophoresis and electro-blotted onto Immobilon P transfer

membrane (Millipore, Billerica, MA, USA). OCRL1 was revealed using polyclonal antibodies raised in rabbit against the N-terminal region (amino-acids 18-217) and a peroxidase-labelled mouse anti-rabbit detection system. As control we used the signals obtained using polyclonal antibodies raised against the β-subunit of the mitochondrial ATPase. The amount of OCRL1 present in each sample was determined by quantitative western blot analysis using a ChemiDoc XRS apparatus and the Quantity One® software (BioRad, Hercules, CA, USA), after correction of protein loading by Coomassie blue staining.

## Results

### Mutation analysis

We have identified a mutation in the *OCRL1* gene in 130 out of the 175 families included for Lowe's syndrome and in 6 out of the 41 Dent disease families. 51 of these mutations were novel (Table 1) with four of them identified in patients affected by Dent-2 disease. Extensive genomic and cDNA sequencing analysis led to the correction of boundaries of exons 8, 9, 11, 12, 13, 14 in reference to those reported in the Lowe Syndrome Mutation Database[28] and to the use of a cDNA numbering based on the use of initiation codon in exon 1 (Supplementary Table S1).

Near to 200 mutations have been identified so far in the *OCRL1* gene in association with Lowe syndrome or Dent-2 disease (Supplementary Table S2). Frameshift, splicing or non-sense mutations leading to a premature termination of the protein represented 63% of the mutations whereas missense mutations and genomic deletion accounted respectively for 33% and 4% of the molecular variations. No noticeable differences in this distribution were noticed when comparing Lowe syndrome or Dent-2 Disease. 33 recurrent mutations were identified and accounted for the disease in 41% of the genotyped families. 17 mutations have been

reported in more than 3 families (n=3: p.Ile274Thr, p.Arg318Cys, p.Arg334Stop, p.Gln388Stop, p.Arg822Stop; n=4: c.940-11G>A, p.Gly421Glu, p.Arg500Stop, p.Arg541Stop, c.1694_1695insCCTT, c.2360_2361delTG, p.Arg844Stop; n=5: p.Arg663Stop, p.Arg695Stop , p.Arg810Stop; n=8: p.Arg500Gln; n=10: c.2581G>A). As it could be inferred from data presented in supplementary Table S2, none of these mutations appeared to be population specific.

Genetic investigation of 98 mothers of patients affected by Lowe syndrome showed the occurrence of a *de novo* mutation in 36 cases (37.2 %) whereas 3 cases of mosaicism were characterized in our panel of genotyped families indicating that, although not frequent, mosaicism must be taken in account for genetic counselling.

As shown in Figure 1, 53 out of the 66 missense mutations reported to date mapped to the PI(4,5)$P_2$ phosphatase domain spanning exons 9-15. They involved amino-acids defined as major determinants for specificity of the inositol polyphosphate 5-phosphatase activity [Whisstock et al., 2000; Tsujishita et al., 2001]. The remaining missense mutations were identified in the C-terminal ASH-RhoGAP-like domain spanning exons 16 to 23 [Peck et al., 2002; Faucherre et al., 2003, Choudhury et al., 2005; Hyvola et al., 2006; Erdmann et al., 2007] and involved amino acids located in well conserved domains (supplementary Figure S1). Noticeably, no missense mutations have been identified so far in exons 1 to 8.

Frameshift mutations or nonsense mutations leading to premature termination of the protein have been characterized in all exons but exons 2 and 3 (Fig 1). While nine substitutions affected directly the consensus acceptor or donor sites at the exon-intron junctions, seven additional intronic mutations induced abnormal splicing: c.40-14A>G, c.238+4701G>A, c.940-11G>A, c.1467-3C>G, c.1879+5G>A, c.1880-5del16, c.2469+2_6delinsA, c.2581+4A>G. Transcript analysis showed that 3 substitutions affecting the last nucleotide of exons 9, 14 and 22: c.824G>C, c.1466G>A, c.2581G>A affected the

splicing. Thus, the p.Gly275Ala, p.Ser489Asn, p.Ala861Thr mutations, initially reported as missense mutations [Kawano et al., 1998; Monnier et al., 2000] led thus primarily to a premature termination of the protein.

Noticeably, while frameshift and nonsense mutations associated with Lowe syndrome concentrated in exons exons 8 to 23, all frameshift and nonsense mutations causative of Dent 2 disease have been characterized so far only in the first seven exons.

**Functional expression of mutations**

*mRNA expression*

As shown in Figure 2 no significant differences in the level of mRNA expression of the *OCRL1* gene could be evidenced in patients harbouring a missense mutation when compared to controls. At difference, patients harbouring stop, frameshift or splicing mutations showed a very low mRNA content that indicated either a decreased transcription or more likely a mRNA instability resulting from a non sense mediated decay mechanism. No difference was observed between patients affected by Lowe syndrome or Dent-2 disease. Noticeably, one patient harbouring a c.[40-14A>G] splicing mutation showed a normal amount of mRNA (Figure 2, arrow). As shown in panel A of the figure 3, the c.40-14A>G mutation resulted in the production of two abnormal transcripts. Sequencing analysis showed that whereas both transcripts could be amplified and quantified, they both lacked the 3' end of exon 1 that contains the ATG initiation codon (supplementary Fig S2).

*PIP2ase activity*

PI (4,5)$P_2$ phosphatase activity was measured in 12 patients harbouring a missense mutation and in 11 patients harbouring a nonsense or a frameshift mutation that led to a premature termination of the protein. As presented in table 2 the enzymatic activities measured in patients with either a missense mutation or a mutation leading to a premature termination of the protein showed a mean inhibition of 85-90% when compared to PI (4,5)$P_2$ phosphatase activity determined in fibroblasts originating either from healthy individuals or from patients addressed for a suspicion of Lowe syndrome and for whom no mutation had been identified in the *OCRL1* gene. No significant differences between the residual activities measured in fibroblasts harbouring a missense mutation and those harbouring a nonsense or frameshift mutation were evidenced. Likewise no differences of PI (4,5)$P_2$ phosphatase activities could be evidenced between Lowe syndrome or Dent-2 patients (not shown). Residual activities measured in mutant fibroblasts were likely to correspond to non specific hydrolysis of PI (4,5)$P_2$ by other inositol polyphosphate phosphatases present in fibroblasts since they were also determined in cells fully devoid of OCRL1 as a consequence of a genomic deletion of the *OCRL1* gene [Lin et al., 1997; Monnier et al., 2000; this paper].

*OCRL1 protein analysis*

The amount of OCRL1 was estimated in total cell extracts using either the total protein amount or the β-ATPase signals for normalisation. As shown in Figure 4, patients harbouring a missense mutation in the *OCRL1* gene (lanes 2-6) had a markedly reduced content of OCRL1 when compared to controls (lanes 1, 7, 8, 12). Two bands were revealed by antibodies, the upper band at ≈ 105 kDa corresponds to the native OCRL1 while the lower band at ≈ 75 kDa corresponds to a proteolytic product [Lichter-Konecki et al., 2006]. As expected, cells harbouring stop and frameshift mutations and characterized by a low mRNA content showed an almost complete absence of OCRL1 (lanes 9,10,11). For quantification

studies of OCRL1 presented in table 3, both bands were taken in account. Unexpectedly, most missense mutations resulted in a drastic decrease of the OCRL1 content although their mRNA content was unaffected (Fig 2). The decrease in OCRL1 was observed whether missense mutation mapped to the central phosphatase domain or to the C-terminal Rho-Gap domain (Fig 4). This indicated that missense mutations not only could affect the catalytic properties of OCRL1 but also could affect the stability of the protein. Noticeably, expression of the c.40-14A>G resulted in the presence of a protein doublet at ≈ 80 kDa with no detectable bands at 100 or 75kDa (Fig 3, panel B, lane 2).

**Clinical expression of mutations**

All but two of the 124 male patients that presented with classical features of the disease i.e. congenital bilateral cataract, central nervous system symptoms and kidney troubles harboured a mutation in the *OCRL1* gene. However as no cDNA analysis could be performed for these two patients, we cannot exclude the presence of a mutation not detected by genomic sequencing. At contrast, no mutation in the *OCRL1* gene was identified in the 36 additional male patients presenting with an incomplete panel of oculocerebrorenal symptoms although highly suggestive of Lowe syndrome. When tested $PI(4,5)P_2$ phosphatase activity also excluded the diagnosis of Lowe syndrome in these patients.

6 out of the 41 patients included on the basis of diagnosis of a Dent disease not associated to the *CLC5* gene carried a mutation of the *OCRL1* gene. None of these 6 patients had ocular or CNS symptoms.

A few mutations that mapped to the same amino acid or to amino acids located at close vicinity were identified in patients addressed either for diagnosis of Lowe syndrome or Dent disease (Table 4). The p.Ile274Thr initially described in a patient presenting with the

phenotype of Dent disease [Utsch et al., 2006] was identified in two families, one presenting with a classical Dent disease and one diagnosed as a Lowe syndrome family in which the two affected brothers had mental retardation and developmental with one of them having a congenital cataract. Likewise, the p.Arg318Cys mutation previously reported in 2 families with Dent disease [Hoopes et al., 2005; Sekine et al., 2007] was identified in a patient presenting with Dent disease and in another patient initially diagnosed as Lowe syndrome patient on the basis of renal and CNS symptoms although ocular symptoms at 24 y of age were restricted to a severe myopia. Three mutations mapped to amino acids closely located in the Rho-GAP domain i.e. p.Ala797Pro, p.Pro799Leu and p.Pro801Leu. While all the three mutations were associated with proximal tubulopathy, only two of them (p.Ala797Pro and p.Pro801Leu) were associated with congenital cataract and SNC symptoms, typical features of the Lowe syndrome.

## CONCLUSIONS AND DISCUSSION

A major consequence of mutations affecting the *OCRL1* gene was the loss of PI(4,5)$P_2$ase activity regardless of the Lowe or Dent-2 clinical phenotypes associated with mutations in patients and regardless of the pathogenic mechanisms.

Nonsense mutations, genomic deletions, frameshift or splicing mutations leading to the occurrence of a premature termination codon represent 2/3 of the OCRL1 mutations identified to date. Expression studies of these mutations indicated that all but one of the mutations tested resulted in an almost complete absence of mRNA and protein (Fig. 2 and table 3).

Interestingly, the splicing c.40-14G>A mutation allowed an apparently normal quantitative production of mRNA. However and as shown in Fig. 3 (panel A) the mutation led

to the synthesis of two abnormal transcripts lacking the normal initiation codon in exon 1 (see supplementary Fig. S2). As shown in panel B of Fig. 3 two peptides with a molecular weight close to 80 kDa were evidenced in fibroblasts harbouring the c.40-14 A>G mutation. Based on bioinformatics analyses, it has been recently proposed that a differential splicing of the *OCRL1* gene associated with the possible use of an alternative initiation of translation in exon 8 that would allow the synthesis of a shortened OCRL1 might occur in different tissues and at different developmental stages [Schrimpton et al., 2009]. Remarkably, frameshift and nonsense mutations associated so far with Dent-2 disease were exclusively identified in the first 7 exons of the OCRL1 gene while all frameshift and nonsense mutations causing Lowe syndrome mapped to exons 8-23 (Fig 1). There are only three methionyl residues in the sequence spanning exon 3 to 10 at position 158, 187 and 206, respectively in exons 7 and 8, that will keep the OCRL1 reading frame. As suggested by Schrimpton [Schrimpton et al., 2009], use of these methionyl residues as alternative initiation codons will allow the synthesis of 85.425 kDa, 82.159 kDa and 79.976 kDa peptides respectively, molecular weights compatibles with the observed bands at ≈ 80kDa in Fig 3. Such proteins will lack the PH domain and a major clathrin binding site [Mao et al., 2009] and are likely to display modified interactions with membranes and other proteins.

While most missense mutations mapped to the phosphatase domain of OCRL1 spanning exons 9-15, 14 missense mutations or in-frame deletion have been identified in the C-terminal ASH (ASPM, SPD-2, Hydin)-RhoGAP-like domain spanning exons 16 to 23 (Fig 1). Eight mutations mapped to the ASH/Rab binding domain spanning exons 17-19 (amino acids 539-752) close to residues that have been shown to modulate Rab binding and targeting of OCRL1 to Golgi and endosomes [Hyvola et al., 2006]. The six missense mutations in exons 20-23 affected amino acids that mapped to a region in close interaction with the ASH domain [Erdman et al., 2007]. Mutations in exon 21 clustered to a domain showing significant

homologies with GAP related proteins (Supplementary Fig S1). Although interaction with Rac 1 a member of the Rho family has been reported [Erdman et al., 2007] and low Rac GAP activity measured *in vitro* [Faucherre et al., 2003], whether OCRL1 functions as a GAP *in vivo* is still debated [Lowe, 2005]. Expression studies of the p.I768N and p.A797P mutations in COS7 cells suggested that the Rho-GAP like domain is important for the enzymatic activity of OCRL1 while being catalytically inactive by itself and showed that this domain could also interact with small G-proteins of the Arf family [Lichter-Konecki et al., 2006]. Therefore and as suggested by their effect on activity and OCRL1 content, these last two residues might play a critical role for the folding of the protein. The ASH-RhoGAP-like domain has been recently shown to interact with the Rab5 effector APPL1 [Erdmann et al., 2007; McCrea et al., 2008] and with Ses1 and Ses2, two closely related endocytic proteins [Swan et al., 2010]. Isothermal titration calorimetry studies performed using peptides corresponding to the interacting domains of Ses1 and APPL1 with OCRL1 suggested that binding of Ses and APPL1 are mutually exclusive and abolished by the same mutations at positions 591, 634, 799, 801 in the ASH-RhoGAP-like domain that also disrupt APPL1 binding. This suggested that Lowe syndrome and Dent disease might result from perturbations at multiple sites within the endocytic pathway [Swan et al., 2010]. Noticeably, most variants affected amino acids conserved among OCRL1 species and INPP5B but much less conserved among other polyphosphate phosphatases. This may suggest that interaction with Rab proteins is a specific feature of OCRL1 and INPP5B.

The decrease of the PI(4,5)$P_2$ase activity in mutant cells harbouring a missense mutation was usually associated with a deleterious effect of the missense mutations on phosphatase activity, whether they mapped to the catalytic PI(4,5)$P_2$ase domain or to the Rho-GAP domain [Lin et al., 1997., Hyvola et al., 2006., Schrimpton et al., 2009]. However and as illustrated in Fig. 4 and Table 3, the consequence of missense mutations is mostly a decrease

of the amount of OCRL1 present in the cell. This is likely to result from an abnormal processing of the protein since OCRL1 mRNA levels were normal in the cells harbouring these missense mutations (Fig. 2). The protein degradation might be the consequence of the activation of endoplasmic reticulum-associated degradation (ERAD) or of tunfolded protein response (UPR) as an answer to the accumulation of unfolded or misfolded mutant OCRL1 in the endoplasmic reticulum [Schröder and Kaufman., 2005]. Conclusions of functional studies based on expression mutant OCRL1 in cellular models must thus take in account protein degradation processes when analysing the physiological relevance of the different mutations. Nevertheless, a small amount (20-30%) of OCRL1 was detected in a few mutant cells although PI(4,5)$P_2$ase activity was almost totally absent. In these situations, the overall decrease of PI(4,5)$P_2$ase activity is likely to result from two processes: a decrease of the amount of OCRL1 through protein degradation and a catalytic inactivation. A few missense mutations e.g. p.Phe242Ser, p.Ala797Pro resulted in the presence of both a small amount of protein and a residual Pi$P_2$ase activity. Noticeably, although the Lowe patients harbouring these mutations presented with classical eye and kidney features, their neurological symptoms were less severe and their degree of mental retardation was moderate. This may reflect a differential dependence of tissues or of developmental stages toward PI(4,5)$P_2$ homeostasis. Differences in clinical expression that were observed between patients harbouring different mutations might thus reflect variability in protein synthesis and/or catalytic properties in the different tissues depending on the nature of the mutation beside variability of the individual genetic background.

Mutations of the *OCRL1* gene have been associated with two distinct clinical phenotypes i.e. Lowe syndrome and Dent disease. Although apparently undistinguishable from the Dent phenotype associated to the *CLCN5* gene, Dent phenotype associated with OCRL1 mutations has been referred as Dent-2 disease to specify the genetic cause of the

disease and may represent a mild variant of Lowe syndrome [Bökenkamp et al., 2009]. Accordingly, it has been proposed that a phenotypic continuum exists between Dent-2 disease and Lowe syndrome. This continuum was not only observed between patients harbouring different OCRL1 mutations but also occurred between patients harbouring the same mutation as presented in table 4. Clinical phenotypes range from patients affected by severe Lowe syndrome with typical ocular, neurological and renal features to Dent-2 patients presenting only with renal impairment and comprise atypical forms of Lowe syndrome presenting with incomplete eye symptoms or moderate neurological troubles. Presence of a residual PI(4,5)$P_2$ase activity has been documented during this study in patients presenting usually with a moderate clinical expression. However, it must be noted that ocular and neurological symptoms were documented even in Lowe patients showing a residual PI(4,5)$P_2$ase activity up to 20% of the normal values in their fibroblasts. On the other hand, it must be kept in mind that mild and severe presentations of Lowe syndrome together with pure real forms of Dent disease were associated with a complete loss of the OCRL1 enzymatic activity. A possible explanation of this clinical variability might be the presence of modifying factors (compensatory phosphatases, interacting proteins…) whose expression will depend on the genetic background of the different patients. The main clinical difference observed between two brothers presenting with a Lowe syndrome and harbouring the same p.Ile274Thr mutation e.g. a documented unilateral cataract for the youngest and no signs of cataract for the eldest one would thus likely result from such modifying factors. Along this line we have investigated a possible role of INPP5B, a phosphatase. No significant variations in the INPP5B content could be evidenced when comparing fibroblast extracts originating from healthy controls or patients affected either by Lowe syndrome or Dent-2 disease. This was in agreement with our data showing that no significant differences of the PiP2-ase activity could be evidenced between Lowe syndrome and Dent-2 disease patients (table 2).

The report of truncated forms of OCRL1 that might result from the use of alternative initiation codons open also new perspectives to explain clinical variability associated with OCRL1 mutations. However these investigations were performed on skin fibroblasts and using antibodies that may not recognize all forms of truncated or alternatively spliced OCRL1. It will be very important to extend these studies to other cell types that are more affected by the disease e.g. renal and ocular cells and to use a larger panel of antibodies directed against different domains of the protein in order to gain new insights regarding the differential pathological mechanisms leading to the different clinical phenotypes.



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## LEGENDS OF FIGURES

Figure 1. Mapping of mutations associated with Lowe syndrome and Dent's disease in the *OCRL1* gene.

Exons, including the alternatively spliced exon 18a, are numbered 1 to 23 and are drawn to scale at the exception of the non-coding sequence of exon 23. The catalytic phosphatase domain is indicated in black, the PH domain in hatched, the ASH-Rab binding domain in light grey, the Rho-GAP like domain as a punctuated pattern and the 5' and 3' untranslated regions are shown in dark grey. LIDIA and LIDLE refer to motives involved in clathrin binding [Mao et al., 2009] and FEDNF to amino-acids involved in AP-2 clathrin adaptator binding [Ungewickell et al., 2004]. Frameshift and nonsense mutations are indicated using their cDNA numbering above the schematic representation of the cDNA while missense mutations and in frame deletions are presented below the cDNA and designated by their protein numbering. Mutations indicated in bold correspond to nonsense mutations. Stars refer to intronic mutations leading to splicing defects. Horizontal bars indicated gross genomic deletions.

Figure 2. Quantification of mRNA OCRL1 expression in patients harbouring a mutation of the *OCRL1* gene.

OCRL1 and GAPDH mRNAs were isolated from fibroblasts and quantified as described in Material and Methods. Horizontal black bar represent the mean and upper and lower boxes the 2 SD limits. Arrow points to the ratio value measured in a patient affected by Dent Disease and harbouring a c.40-14G>A mutation.

Figure 3. mRNA and protein expression of the c.40-14 A>G mutation.

Panel A. cDNA obtained from fibroblasts harbouring the c.40-14A>G mutation was amplified using primers allowing amplification of a sequence encompassing exons 1, 2 and 3. Amplification products were analyzed using a 2% agarose gel. Amplification of the mutant cDNA (lane 1) generated two bands respectively long of 288 and 391 bp (arrows) while amplification of the normal sequence (lane 2) generated a 488 bp fragment as expected.
Panel B. Western blotting of fibroblast extracts was performed as described in Material and Methods. Lanes 1, 4: control fibroblasts; lane 2: c.40-14A>G mutant fibroblasts; lane 3: p.Ile274Thr mutant fibroblasts.

Figure 4. Western blot analysis of OCRL1 in control and patient fibroblasts.
Fibroblasts extract (25 µg) prepared as described in Material and Methods was loaded in each lane. Upper panel: western blot analysis using polyclonal antibodies directed againts OCRL1. Lane M: molecular weight markers, lanes 1, 7, 8, 12 : control patients, lane 2: p.Glu468Gly, lane 3: p.Arg318Cys, lane 4: p.Asn373Tyr, lane 5: p.Pro801Cys, lane 6: p.Pro799Leu, lane 9: p. Arg810Stop, lane 10: c.2469+2T>G, lane 11: c.825-2A>G. Lower panel: protein loading in the different lanes was demonstrated by a western blot analysis of the same blot with the use of antibody against the β subunit of the mitochondrial ATPase .

Figure S1. Conservation of residues located in the phosphatidyl-inositol phosphatase catalytic domain and in the Rab and Rho-GAP domains of OCRL1 and related proteins.
Amino acids alignment is given with identical residues shown by dashes. Mutated residues are shown in bold. Numbering of the amino-acids refer to the human OCRL1 sequence (ENSP00000360154). Upper amino acids represent the missense mutations identified in patients affected by Lowe syndrome and Dent disease (underlined character). Sequences were obtained from SwissProt and Genbank with the following accession number. OCRL1: H.

sapiens Q01968 , M. musculus Q6NVF0, C. elegans NM 060183, D. melanogaster NP 569962; INPP5B: NP005531; INPPL1: NP001558; SHIP: NP001017915; Synaptojanin: NP003886; ARHGAP10: AAI09030; RHOGAP4: NP001657; Oligophrenin: O60890; RACGAP1: AAH32754 .

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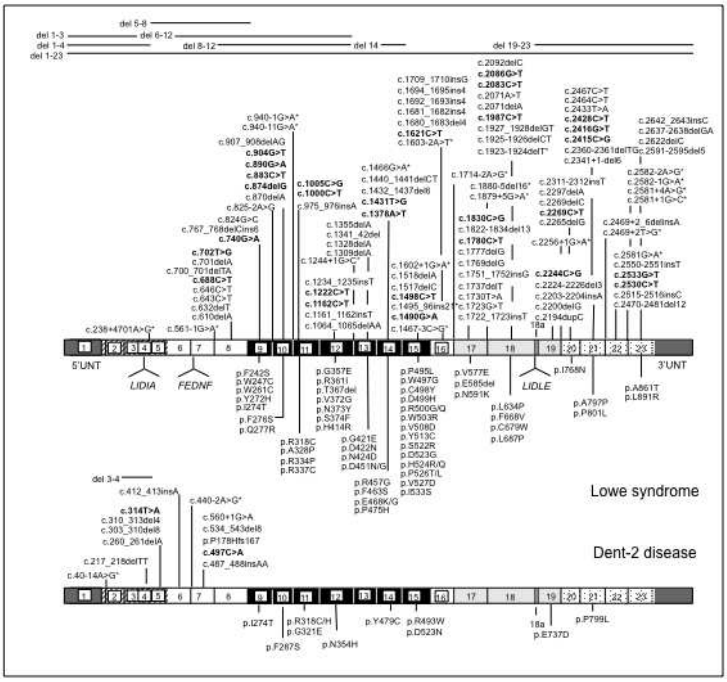
Figure S2. Schematic representation of the splicing consequences of the c.40-14G>A mutation.

Element involved in the splicing (donor and acceptor sites) of normal and mutant exons 1, 2 and 3 are indicated in the sequences (bold character). The c.40-14A>G mutation (underlined character) revealed a cryptic donor site in exon 1 located in position -82 that was used for both mutated transcripts. In mutant transcript #1 splicing used a cryptic acceptor site of intron 1 at position -24 while exon 2 was completely skipped in mutant transcript #3. This representation was based on sequencing data obtained after cloning of the different transcripts that have been evidenced.




275x190mm (72 x 72 DPI)

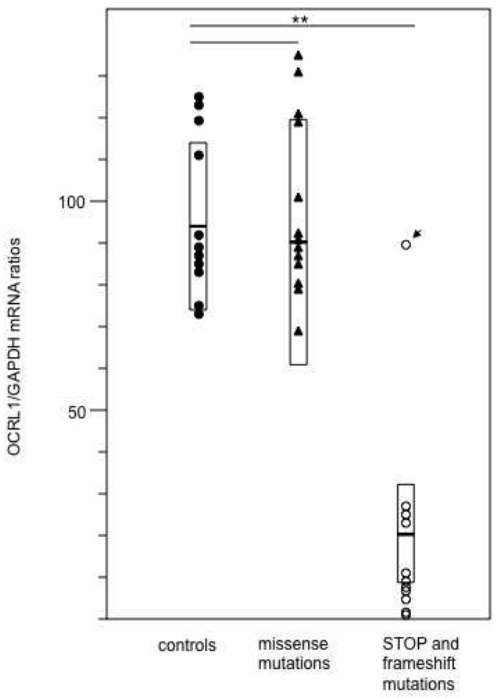



Figure 2
254x190mm (72 x 72 DPI)

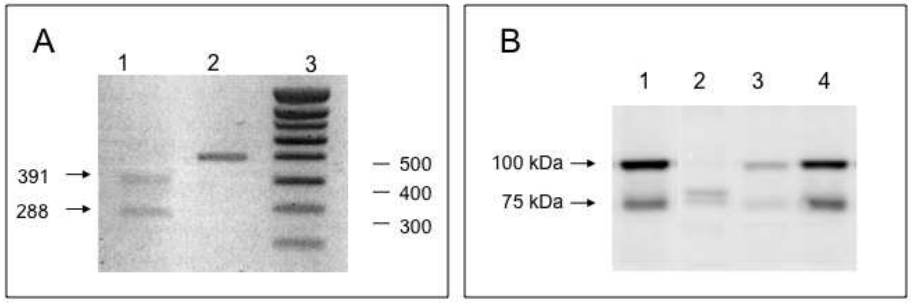



Figure 3
254x190mm (72 x 72 DPI)

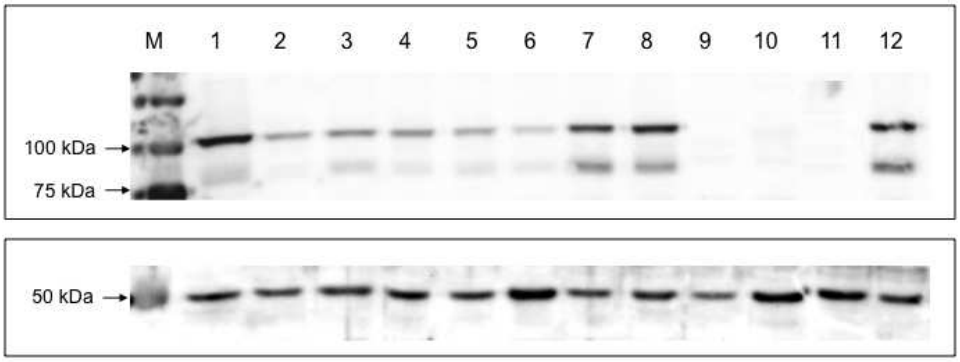



Figure 4
254x190mm (72 x 72 DPI)

| Patient ID | Exon | Nucleotide change[a] | Protein change[b] | Enzyme activity[c] | Disease status | Age[d] | Ocular symptoms[e] | | Neurological symptoms[e] | | | Renal symptoms[e] | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | CC | G | NH | DD | MR | FS | PT | RF | AA | LMWP | P | Ca |
| 05den10 | 1 | c.40-14A>G | Splicing defect | 1.6 | Dent | 7 | - | | - | | | | | | + | | + | |
| 09ls009 | i1 | c.238+4701A>G | Splicing defect | | Lowe | 7 | + | | | + | | | + | | | + | | |
| 04ls15 | 8 | c.632delT | p.Val211AspfsX38 | | Lowe | 6 | + | + | + | + | +/- | | | | + | | + | |
| 06ls37 | 8 | c.688C>T | p.Arg230X | | Lowe | 2 | + | | + | + | | | + | | + | | | |
| 05ls26 | 8 | c.702T>G | p.Tyr234X | | Lowe | 1 | | | | | | | | | | | | |
| 02ls42 | 9 | c.725T>C | p.Phe242Ser | 2.6 | Lowe | 30 | + | + | + | + | + | | + | | | + | | + |
| 02ls25[f] | 9 | c.821T>C | p.Ile274Thr | | Lowe | 43 | - | | | + | +/- | | | + | | + | | |
| 02ls26[f] | | | | | Lowe | 34 | + | | | + | +/- | | | + | | + | | |
| 08ls008 | i9 | c.825-2A>G | Splicing defect | | Lowe | 17 | + | | | + | + | | + | | | + | | |
| 06ls59 | 10 | c.830A>G | p.Gln277Arg | | Lowe | 7 | + | | | + | + | | + | | + | + | + | |
| 06ls54 | 11 | c.952C>T | p.Arg318Cys | | Lowe | 23 | - | - | + | + | + | + | + | + | | + | + | |
| 07den01 | | | | | Dent | 7 | - | | | - | | | | | + | + | | |
| 10ls011 | 11 | c.1005C>G | p.Tyr335X | | Lowe | 5 | + | - | + | + | + | | + | | | + | + | |
| 06ls32[g] | 11 | c.1009C>T<br>c.1082G>T | p.Arg337Cys<br>p.Arg361Ile | 1.4 | Lowe | 1 | + | | + | | | | | | | + | + | |
| 04den01 | 12 | c.1060A>C | p.Asn354His | | Dent | 27 | - | | - | | | | | | | | + | |
| 98ls44 | 12 | c.1115T>G | p.Val372Gly | | Lowe | 22 | + | + | | + | | + | + | | | | | |
| 06ls15 | 12 | c.1117A>T | p.Asn373Tyr | 1.4 | Lowe | 13 | + | | + | + | + | + | + | | + | + | | |
| 98ls48 | 12 | c.1121C>T | p.Ser374Phe | | Lowe | 3 | + | | + | + | | + | + | | + | + | + | |
| 10ls016[f] | 12 | c.1241A>G | p.His414Arg | | Lowe | 2 | + | + | + | + | + | + | | | | + | + | + |
| 10ls017[f] | | | | | Lowe | 5 | + | + | + | + | + | + | | | | + | + | + |
| 09ls014 | 13 | c.1341_1342delCT | p.Leu448GlufsX17 | | Lowe | 1 | + | | + | | | + | | | | + | + | |
| 03ls38 | 13 | c.1351G>A | p.Asp451Asn | | Lowe | 30 | + | + | | + | | + | + | | | | | |
| 00ls37 | 13 | c.1355delA | p.Gln452ArgfsX1 | | Lowe | 12 | + | | | + | | | | | | | | |
| 00ls03 | 14 | c.1369C>G | p.Arg457Gly | 0,6 | Lowe | 20 | + | | | + | + | + | | | | | | |
| 06ls17 | 14 | c.1378A>T | p.Lys460X | | Lowe | 1 | + | + | + | | + | + | + | | + | + | + | |
| 03ls35 | 14 | c.1402G>A | p.Glu468Lys | | Lowe | 5 | + | | + | | + | + | + | | | + | + | |
| 07ls20 | 14 | c.1403A>G | p.Glu468Gly | | Lowe | 5 | + | | + | + | | + | | | | + | + | |
| 04ls11 | 14 | c.1440-1441delCT | p.Asp463AspfsX1 | | Lowe | 0.1 | + | + | + | | | + | | | | + | + | |
| 06ls19 | i14 | c.1467-3C>G | Splicing defect | | Lowe | 7 | + | | | | | | | | + | | + | |
| 02ls50 | 15 | c.1484C>A | p.Pro495Leu | 1.8 | Lowe | 3 | + | | + | | | | + | | | + | | |
| 04ls05 | 15 | c.1495G>C | p.Asp499His | | Lowe | 5 | + | | + | + | | + | | | | | | |
| 06ls18 | 15 | c.1507T>C | p.Trp503Arg | | Lowe | 0.4 | + | + | + | + | | | | | + | | | |
| 05ls21 | 16 | c.1681_1682insGACT | p.Phe561X | | Lowe | 2 | + | + | + | + | + | + | | | + | | + | |
| 00ls29 | 16 | c.1692_1693insCCTT | p.Leu565ProfsX11 | | Lowe | 12 | + | + | | + | + | + | | | | | | |
| 07ls20 | 17 | c.1773C>A | p.Asn591Lys | | Lowe | 13 | + | + | + | + | + | | | | + | + | | |
| 03ls23 | 17 | c.1780C>T | p.Gln594X | | Lowe | 0.9 | + | | + | + | + | + | | | | | + | |
| 04ls21 | i17 | c.1879+5G>A | Splicing defect | 0.3 | Lowe | 32 | + | | | | +/- | | | | | | | |

| | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 00ls23 | 18 | c.1927_1928delGT | p.Val643AsnfsX8 | 1.8 | Lowe | 0.3 | + | | + | + | | | + | | + | + | | |
| 02ls35 | 18 | c.2086G>T | p.Glu696X | | Lowe | 20 | + | | | + | + | | + | | | + | | + |
| 07ls10 | 18 | c.2092delC | p.Pro681LeufsX66 | | Lowe | dead | + | | + | | | | | | | | | |
| 08ls011 | 19 | c.2194dupC | p.Leu732PfsX38 | | Lowe | 1 | + | + | + | + | + | + | + | | | + | | |
| 02ls11 | 19 | c.2200delG | p.Val734PhefsX8 | | Lowe | 6 | + | | + | | | + | | | | | | |
| 08ls15 | 19 | c.2224_2226delGTA | p.Val742del | | Lowe | 10 | + | + | + | + | + | + | + | + | + | + | + | + |
| 98ls54 | 20 | c.2269C>T | p.Gln757X | | Lowe | 23 | + | | + | + | + | +/- | + | + | + | + | + | |
| 05ls29 | 20 | c.2311-2312insT | p.Cys771LeufsX8 | | Lowe | 7 | + | | + | + | + | + | | | | + | + | |
| 09ls001 | | | | | Lowe | 2 | + | | + | + | | | + | | | + | | |
| 04ls17 | 21 | c.2464C>T | p.Arg822X | | Lowe | 2 | + | + | + | + | | | + | | | + | | |
| 05ls17 | i21 | c.2469+2T>G | Splicing defect | 0.3 | Lowe | 1 | + | | + | + | +/- | | + | + | | + | | |
| 01ls19 | i22 | c.2581+1G>C | Splicing defect | | Lowe | 1.5 | + | + | | | | | | | | | | |
| 03ls14 | i22 | c.2582-1G>A | Splicing defect | | Lowe | 23 | + | | | | + | | + | | | + | | |
| 06ls65 | i22 | c.2582-2A>G | Splicing defect | | Lowe | 6 | + | | + | + | + | + | + | | + | + | | |
| 08ls03 | 23 | c.2591_2595del6 | p.Gln879_Thr880>HisfsX4 | | Lowe | 28 | + | + | + | + | + | | + | | + | + | | |
| 02ls08 | 23 | c.2637_2638del | p.Gln862HisfsX3 | | Lowe | 12 | + | | | | + | | | | | | | |
| 09ls013 | 23 | c.2672T>G | p.Leu891Arg | | Lowe | 10 | + | | + | + | | | + | | + | | | |
| 06den06 | 3-4 | del exon 3-4 | Frameshift deletion | | Dent | 9 | - | | - | - | - | | + | | | + | | + |
| 00ls05 | 19-23 | del exons 19-23 | Frameshift deletion | 1.8 | Lowe | 30 | + | | + | + | + | + | | | | | | |

[a] Numbering was based on the cDNA sequence wih +1 corresponding to the A of the ATG initiation codon of translation in the reference sequence.
[b] Expected consequences of the mutations with codon 1 corresponding to the ATG initiation codon of translation.
[c] Enzyme activity measured in controls was N = 9.6 +/- 2.3 nanomoles PI(4,5)$P_2$ hydrolyzed/min/mg
[d] Age at the time of the molecular investigation
[e] CC: congenital cataract, G: glaucoma, NH: neonatal hypotonia, DD: developmental delay, MR: mental retardation, FS: Fanconi syndrome, PT : proximal tubulopathy, RF: renal failure, AA: amino-aciduria, LMWP: low molecular weight proteinuria; P: hypophosphatemia/hyperphosphaturia, Ca : hypercalciuria.
(+) sign present, (-) sign absent, ( ) sign not documented at the time of the last examination
[f] Patients 02ls25 and 02ls26, and 10ls06 and 10ls017 are brothers
[g] Variants c.1009C>T and c.1082G>T are on the same allele

**Table 1 : Novel mutations in the OCRL1 gene**

| | enzyme activity [a] (nanomol $PI(4,5)P_2$ hydrolyzed $mg^{-1}$ $min^{-1}$) (+/- SEM) |
|---|---|
| Patients with missense mutations[b] | 1.39 +/- 0.99 (n = 12) |
| Patients with nonsense or frameshift mutations[b] | 1.54 +/- 0.63 (n = 11) |
| Patients with no OCRL1 mutation identified[c] | 9.43 +/- 1.47 (n = 12) |
| Control individuals | 9.62 +/- 2.31 (n=13) |

[a] n value represent the number of independent patients tested, all measurements
[b] 19 patients were affected by Lowe syndrome, 4 presented with Dent-2 disease
[c] these patients were adressed to the laboratory for molecular and biochemical investigations. on the basis of suspicion of Lowe syndrome

**Table 2. $PI(4,5)P_2$ phosphatase activity in patients with OCRL1 mutations**

| | OCRL1 / β ATPase [a] relative units (+/- SEM) |
|---|---|
| Patients with nonsense or frameshift mutations | 0.05 +/- 0.02 (n = 11) |
| Patients with missense mutations | 0.35 +/- 0.14 (n = 12) |
| Control individuals | 1.70 +/- 0.36 (n = 13) |

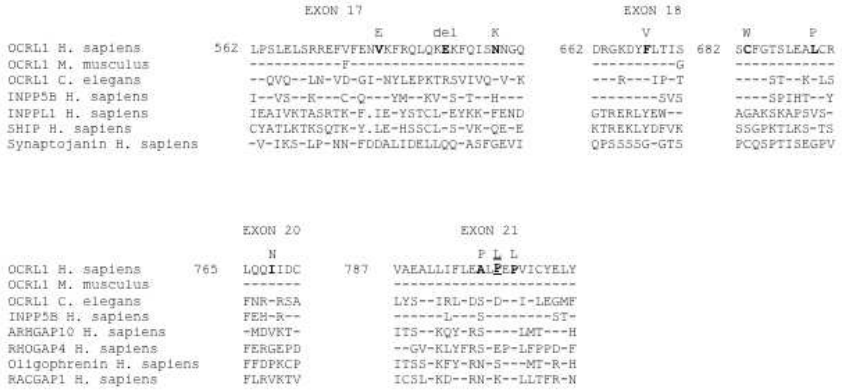
[a] n value represent the number of independent patients tested

**Table 3. OCRL1 content in fibroblasts originating from patients with OCRL1 mutations**

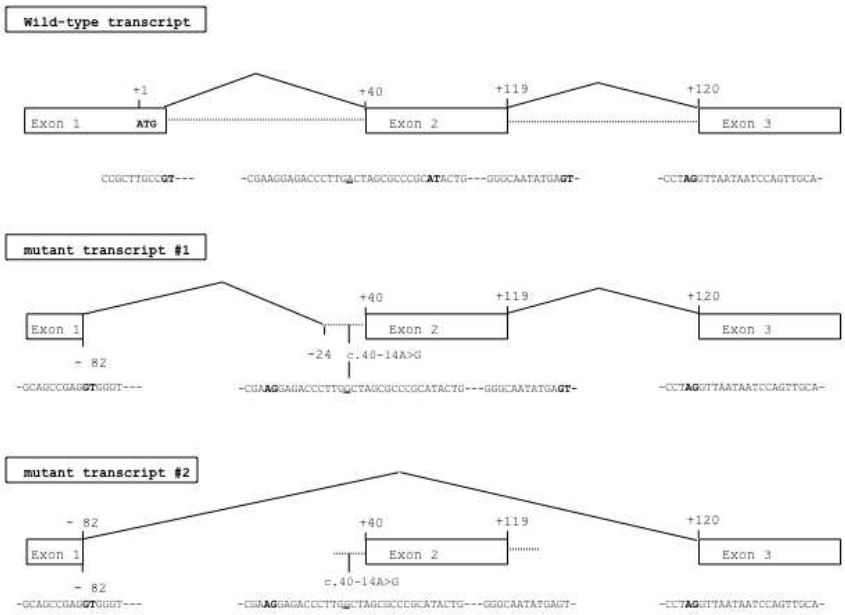
| Patient ID | Clinical diagnosis[a] | Mutation | Age[b] | $PiP_2$ase activity[c] | Ocular symptoms | SNC symptomss | Renal symptoms |
|---|---|---|---|---|---|---|---|
| 98ls18 | Lowe | del exons 1-4 | 3 | | Congenital bilateral cataract | Neonatal hypotonia, convulsions | Fanconi syndrome |
| 06den06 | Dent | del exon 3-4 | 14 | | None | None | LMW proteinuria, hypercalciuria |
| 02ls25[d] | Lowe | p.Ile274Thr | 43 | | None | Moderate mental retardation allowing an autonomous life | Nephrotic syndrome, proteinuria, renal failure |
| 02ls26[d] | Lowe | p.Ile274Thr | 34 | | Congenital unilateral cataract | Moderate mental retardation allowing an autonomous life | Renal failure, proteinuria |
| 05den11 | Dent | p.Ile274Thr | 2 | | None | Developmental delay | Proximal tubulopathy, proteinuria, hypercalciuria |
| 06ls59 | Lowe | p.Arg318Cys | 24 | | Severe myopia | Neonatal hypotonia, severe mental retardation | Fanconi syndrome, proteinuria, hyperphophaturia, renal failure |
| 07den01 | Dent | p.Arg318Cys | 4 | | None | None | Tubular proteinuria, hypercalciuria |
| 98ls80 | Lowe | p.Ala797Pro | 57 | 2.9 | Congenital bilateral cataract | Moderate mental retardation and mild psychomotor troubles | Fanconi syndrome |
| 04den05 | Dent | p.Pro799Leu | 21 | 0.8 | None | None | LMW proteinuria, hypercalciuria, nephrocalcinosis, hyperphosphaturia, aminoaciduria |
| 06ls27 | Lowe | p.Pro801Leu | 1 | 1.6 | Congenital bilateral cataract | Neonatal hypotonia, moderate developmental delay, no psychomotor troubles | Glomerular and tubular proteinuria |

[a] initial clinical diagnosis
[b] age at the time of the molecular investigation
[c] enzyme activity measured in controls was N = 9.6 +/- 2.3 nanomoles $PI(4,5)P_2$ hydrolyzed/min/mg
[d] patients 02ls25 and 02ls26 are brothers

**Table 4. Clinical expression of OCRL1 mutations in patients affected by Lowe syndrome or Dent disease**




Supplementary Figure 1
254x190mm (72 x 72 DPI)




Supplementary Figure 2
254x190mm (72 x 72 DPI)

| exon | | |
|---|---|---|
| | Previous numbering[a] | Corrected numbering[b] |
| 1 | to -13 | -169 to 39 |
| 2 | -12 to 68 | 40 to 119 |
| 3 | 69 to 148 | 120 to 199 |
| 4 | 149 to 187 | 200 to 238 |
| 5 | 188 to 298 | 239 to 349 |
| 6 | 299 to 388 | 350 to 439 |
| 7 | 389 to 509 | 440 to 560 |
| **8** | 510 to 670 | 561 to **722** |
| **9** | 671 to 773 | **723** to 824 |
| 10 | 774 to 888 | 825 to 939 |
| **11** | 889 to 1004 | 940 to **1056** |
| **12** | 1005 to 1193 | **1057** to 1244 |
| **13** | 1194 to 1304 | 1245 to **1356** |
| **14** | 1305 to 1415 | **1357** to 1466 |
| 15 | 1416 to 1551 | 1467 to 1602 |
| 16 | 1552 to 1662 | 1603 to 1713 |
| 17 | 1663 to 1828 | 1714 to 1879 |
| 18 | 1829 to 2064 | 1880 to 2115 |
| **18a** | 2065 to 2088 | 2116 to **2140** |
| **19** | 2089 to 2205 | **2141** to 2256 |
| 20 | 2206 to 2290 | 2257 to 2341 |
| 21 | 2291 to 2418 | 2342 to 2469 |
| 22 | 2419 to 2530 | 2470 to 2581 |
| 23 | 2531 to 4935 | 2582 to 4986 |

[a] Previous numbering [Attree et al.,1992; LSMD, 2010]
[b] Conversion of previous numbering using the ATG start codon of exon 1 (+51 bp), bold numbers correspond to exons for which boundaries have been corrected

**Supplementary table 1. Numbering of exon boundaries**

| Exon Intron | Nucleotide change[a] | Protein change[b] | Enzyme activity N = 1[c] | Protein[d] | Number of patients[e] | Geographical origin[f] | Disease status | References |
|---|---|---|---|---|---|---|---|---|
| 1 | c.40-14A>G | Splicing defect | 0.17 | | 1 | France | Dent-2 | This paper |
| 4 | c.217_218delTT | p.Leu73AspfsX1 | | | 1 | North-America | Dent-2 | Schimpton et al., 2009 |
| i4 | c.238+4701A>G | Splicing defect | | | 1 | UK | Lowe | This paper |
| 5 | c.260_261delA | p.Gln87ArgfsX88 | | | 2 | Turkey | Dent-2 | Utsch et al., 2006 |
| 5 | c.303_310del8 | p.Glu101GludelfsX27 | | | 1 | Japan | Dent-2 | Sekine et al., 2007 |
| 5 | c.310_313delTGTT | p.Cys104X | 0.10 | - | 1 | Germany | Dent-2 | Hoopes et al., 2005 |
| 5 | c.314T>A | p.Leu105X | | | 1 | Italy | Dent-2 | Tosetto et al, 2009 |
| 6 | c.412_413insA | T138 AsnfsX122 | | | 2 | Germany | Dent-2 | Utsch et al., 2006 |
| i6 | c.440-2A>G | Splicing defect | 0.11 | - | 1 | Germany | Dent-2 | Hoopes et al., 2005 |
| 7 | c.487_488insAA | p.K163KfsX2 | 0.09 | - | 1 | Germany | Dent-2 | Hoopes et al., 2005 |
| 7 | c.497C>G | p.Ser166X | | | 1 | North-America | Dent-2 | Schimpton et al., 2009 |
| 7 | | p.Pro178HisfsX167 | | | 1 | Italy | Dent-2 | Tosetto et al, 2009 |
| 7 | c.534_543del | p.Pro178ProfsX3 | | | 1 | North-America | Dent-2 | Schimpton et al., 2009 |
| 7 | c.560+1G>A | p.Met187IlefsX1 | | | 1 | North-America | Dent-2 | Schimpton et al., 2009 |
| i7 | c.561-1G>A | Splicing defect | 0.10 | | 1 | North-America | Lowe | LSMD, 2010 |
| 8 | c.610delA | p.Gln204ThrfsX45[d] | | | 1 | Italy | Lowe | Addis et al., 2004 |
| 8 | c.632delT | p.Val211AspfsX38 | | | 1 | Spain | Lowe | This paper |
| 8 | c.643C>T | p.Gln215X | | | 1 | North-America | Lowe | LSMD, 2010 |
| 8 | c.646delT | p.Ser216LeufsX39 | | | 1 | ni | Lowe | LSMD, 2010 |
| 8 | c.688C>T | p.Arg230X | | | 1 | Australia | Lowe | This paper |
| 8 | c.700_701delTA | p.Tyr234CysfsX21 | | | 1 | ni | Lowe | LSMD, 2010 |
| 8 | c.701delA | p.Tyr234LeufsX15 | | | 1 | ni | Lowe | LSMD, 2010 |
| 8 | c.702T>G | p.Tyr234X | | | 1 | Spain | Lowe | This paper |
| 9 | c.725T>C | p.Phe242Ser | 0.13 | ++ | 1 | France | Lowe | This paper |
| 9 | c.740G>A | p.Trp247X | | | 1 | ni | Lowe | LSMD, 2010 |
| 9 | c.741G>T | p.Trp247Cys | | | 1 | Italy | Lowe | Addis et al., 2004 |
| 9 | c.767_768delCinsAGACTT | p.Ser256_Gly257delinsfsX10 | | | 1 | North-America | Lowe | LSMD, 2010 |
| 9 | c.783G>C | p.Trp261Cys | | | 1 | ni | Lowe | LSMD, 2010 |
| 9 | c.814T>C | p.Tyr272His | | | 1 | North-America | Lowe | LSMD, 2010 |
| 9 | c.821T>C | p.Ile274Thr | | | 1 | France | Lowe | This paper |
| 9 | c.821T>C | p.Ile274Thr | | | 2 | Germany, France | Dent-2 | Utsch et al., 2006 ; this paper |
| 9 | c.824G>C | Splicing defect / p.Gly275Ala | | | 1 | France | Lowe | Monnier et al., 2000 |
| i9 | c.825-2A>G | Splicing defect | | | 1 | French | Lowe | This paper |
| 10 | c.827T>C | p.Phe276Ser | 0.05 | +++ | 1 | Morocco | Lowe | Chabaa et al., 2006 |
| 10 | c.830A>G | p.Gln277Arg | | | 1 | India | Lowe | Sehti et al., 2008 |
| 10 | c.860T>C | p.Phe287Ser | | | 1 | Italy | Dent-2 | Tosetto et al, 2009 |
| 10 | c.870delA | p.Glu290AspfsX2 | | | 1 | ni | Lowe | LSMD, 2010 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 10 | c.874delG | p.Val292X | 0,05 | - | 1 | North-America | Lowe | Lin et al., 1997 |
| 10 | c.883C>T | p.Gln295X | | | 1 | North-America | Lowe | Lin et al., 1997 |
| 10 | c.890G>A | p.Trp297X | | | 1 | Germany | Lowe | LSMD, 2010 |
| 10 | c.904G>T | p.Glu302X | | | 1 | India | Lowe | Sehti et al., 2008 |
| 10 | c.907_908delAG | p.Arg303ArgfsX9 | | | 1 | France | Lowe | Monnier et al., 2000 |
| i10 | c.940-11G>A | Splicing defect | 0,13-0.17 | - | 5 | China, France Norway, Japan | Lowe | Chou et al., 2005 ; this paper |
| i10 | c.940-1G>A | Splicing defect | | | 1 | ni | Lowe | Lin et al., 1998 |
| 11 | c.952C>T | p.Arg318Cys | 0.16-0.18 | +++ | 3 | Germany, France Japan | Dent-2 | Hoopes et al., 2005 ; Sekine et al., 2007 ; this paper |
| 11 | c.952C>T | p.Arg318Cys | | | 1 | France | Lowe | This paper |
| 11 | c.953G>A | p.Arg318His | | | 1 | North-America | Dent-2 | Schimpton et al., 2009 |
| 11 | c.962G>A | p.Gly321Glu | | | 1 | North-America | Dent-2 | Schimpton et al., 2009 |
| 11 | c.975-976insA | p.Leu325LeufsX14 | | | 1 | France | Lowe | Satre et al., 1999 |
| 11 | c.982G>C | p.Ala328Pro | | | 1 | ni | Lowe | Lin et al., 1998 |
| 11 | c.1000C>T | p.Arg334X | | | 3 | Spain, France | Lowe | Satre et al., 1999 ; Monnier et al., 2000 |
| 11 | c.1001G>C | p.Arg334Pro | | | 1 | ni | Lowe | LSMD, 2010 |
| 11 | c.1005C>G | p.Tyr335X | | | 1 | France | Lowe | This paper |
| 11 | c.1009C>T | p.Arg337Cys | 0.15 | | 1 | France | Lowe | This paper |
| 12 | c.1060A>C | p.Asn354His | | | 1 | France | Dent-2 | This paper |
| 12 | c.1064_1014delAA | p.Lys355ArgdelfsX29 | | | 1 | ni | Lowe | LSMD, 2010 |
| 12 | c.1070G>A | p.Gly357Glu | | | 2 | France | Lowe | Monnier et al., 2000 |
| 12 | c.1082G>T | p.Arg361Ile | | | 1 | France | Lowe | This paper |
| 12 | c.1099-1101delACC | p.Thr367del | 0.01 | + | 2 | North-America Spain | Lowe | Lin et al., 1997 ; Satre et al., 1999 |
| 12 | c.1115T>G | p.Val372Gly | | | 1 | France | Lowe | This paper |
| 12 | c.1117A>T | p.Asn373Tyr | 0.15 | ++ | 1 | France | Lowe | This paper |
| 12 | c.1121C>T | p.Ser374Phe | | | 1 | France | Lowe | This paper |
| 12 | c.1123C>T | p.His375Tyr | 0.0 | + | 1 | North-America | Lowe | Kubota et al., 1998 |
| 12 | c.1161_1162insT | p.Gln388SerfsX4 | | | 1 | ni | Lowe | LSMD, 2010 |
| 12 | c.1162C>T | p.Gln388X | 0.12 | - | 3 | North-America Japan, Germany | Lowe | Lin et al., 1997 ; Kubota et al., 1998 ; Röschinger et al., 2000 |
| 12 | c.1222C>T | p.Gln408X | | | 2 | Japan | Lowe | Kubota et al., 1998 |
| 12 | c.1234_1235insT | p.Met412IlefsX4 | | | 1 | India | Lowe | Sehti et al., 2008 |
| 12 | c.1241A>G | p.His414Arg | | | 2 | Kosovo | Lowe | This paper |
| i12 | c.1244+1G>C | Splicing defect | | | 1 | Italy | Lowe | Addis et al., 2004 |
| 13 | c.1262G>A | p.Gly421Glu | 0.05-0.19 | + | 4 | Canada, Switzerland | Lowe | Monnier et al., 2000 ; this paper |
| 13 | c.1264G>A | p.Asp422Asn | | | 1 | ni | Lowe | LSMD, 2010 |
| 13 | c.1270A>G | p.Asn424Asp | | | 1 | France | Lowe | Monnier et al., 2000 |
| 13 | c.1309delA | p.Ser437ValfsX12 | | | 1 | | Lowe | LSMD, 2010 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 13 | c.1341_1342delCT | pLeu448GlufsX17 | | | 1 | France | Lowe | This paper |
| 13 | c.1328delA | p.Asp443AlafsX6 | | | 1 | France | Lowe | Monnier et al., 2000 |
| 13 | c.1351G>A | p.Asp451Asn | | | 1 | France | Lowe | This paper |
| 13 | c.1352A>G | p.Asp451Gly | 0.02 | + | 1 | North-America | Lowe | Lin et al., 1997 |
| 13 | c.1355delA | p.Gln452ArgfsX2 | | | 1 | Australia | Lowe | This paper |
| 14 | c.1369C>G | p.Arg457Gly | 0,06 | +/- | 1 | Canada | Lowe | This paper |
| 14 | c.1378A>T | p.Lys460X | | | 1 | Spain | Lowe | This paper |
| 14 | c.1387T>C | p.Phe463Ser | | | 1 | North-America | Lowe | Lin et al., 1997 |
| 14 | c.1402G>A | p.Glu468Lys | | | 1 | France | Lowe | This paper |
| 14 | c.1403A>G | p.Glu468Gly | | ++ | 1 | France | Lowe | This paper |
| 14 | c.1424G>A | p.Pro475His | | | 1 | China | Lowe | Chou et al., 2005 |
| 14 | c.1431T>G | P.Tyr477X | | | 2 | Italy | Lowe | Addis et al., 2004 |
| 14 | c.1432_1437del6 | p.Lys479_Tyr480del | | | 1 | Germany | Lowe | Röschinger et al., 2000 |
| 14 | c.1436A>G | p.Tyr479Cys | 0.13 | ++ | 1 | Germany | Dent-2 | Hoopes et al., 2005 |
| 14 | c.1440-1441delCT | p.Asp480AspfsX1 | | | 1 | Australia | Lowe | This paper |
| 14 | c.1466G>A | Splicing defect / p.Ser489Asn | | | 1 | France | Lowe | Monnier et al., 2000 |
| i14 | c.1467-3C>G | Splicing mutation | | | 1 | UK | Lowe | This paper |
| 15 | c.1477C>T | p.Arg493Trp | | | 2 | Germany, Japan | Dent-2 | Utsch et al., 2006 ; Sekine et al., 2007 |
| 15 | c.1484C>A | p.Pro495Leu | 0,12 | + | 1 | Belgium | Lowe | This paper |
| 15 | c.1489T>G | p.Trp497Gly | | | 1 | ni | Lowe | Schneider et al., 2001 |
| 15 | c.1490G>A | p.Trp497X | | | 1 | ni | Lowe | LSMD, 2010 |
| 15 | c.1493G>A | p.Cys498Tyr | | | 2 | France | Lowe | Monnier et al., 2000 |
| 15 | c.1495G>C | p.Asp499His | | | 1 | Norway | Lowe | This paper |
| 15 | c.1495-1496ins21 | p.Asp499delinsGRVPATCY | | | 1 | ni | Lowe | Böckenhauer et al., 2008 |
| 15 | c.1498C>G | p.Arg500Gly | | | 2 | France, ni | Lowe | Satre et al., 1999 |
| 15 | c.1498C>T | p.Arg500X | | | 5 | Serbia, France, India | Lowe | Monnier et al., 2000 ; this paper |
| 15 | c.1499G>A | p.Arg500Gln | 0.1 | + | 9 | North-America Japan, Germany, France | Lowe | Lin et al., 1997 ; Kawano et al., 1998 ; Röschinger et al., 2000 ; LSMD, 2010 ; this paper |
| 15 | c.1507T>C | p.Trp503Arg | | | 1 | France | Lowe | This paper |
| 15 | c.1517delC | p.Thr506LysfsX13 | | | 1 | Basque country | Lowe | Monnier et al., 2000 |
| 15 | c.1518delA | pThr506ThrfsX13 | 0.08 | - | 1 | North-America | Lowe | Lin et al., 1997 |
| 15 | c.1523T>A | p.Val508Asp | | | 1 | North-America | Lowe | Lin et al., 1998 |
| 15 | c.1538A>G | p.Tyr513Cys | 0,10 | ++++ | 1 | North-America | Lowe | Lin et al., 1998 |
| 15 | c.1566C>G | p.Ser522Arg | | | 1 | Japan | Lowe | Kubota et al., 1998 |
| 15 | c.1567G>A | p.Asp523Asn | | | 1 | Italy | Dent-2 | Tosetto et al, 2009 |
| 15 | c.1568A>G | p.Asp523Gly | | | 1 | ni | Lowe | LSMD, 2010 |
| 15 | c.1571A>G | p.His524Arg | 0.03 | ++ | 1 | North-America | Lowe | Lin et al., 1997 |
| 15 | c.1572C>G | p.His524Gln | 0.1 | - | 1 | Japan | Lowe | Kawano et al., 1998 |
| 15 | c.1576C>A | p.Pro526Thr | | | 1 | ni | Lowe | LSMD, 2010 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 15 | c.1577C>T | p.Pro526Leu | | | 1 | Germany | Lowe | Röschinger et al., 2000 |
| 15 | c.1580T>A | p.Val527Asp | | | 1 | Italy | Lowe | Addis et al., 2004 |
| 15 | c.1598T>G | p.Ile533Ser | deficient | | 1 | ni | Lowe | LSMD, 2010 |
| i15 | c.1602+1G>A | Splicing defect | 0.17 | + | 2 | Israel, France | Lowe | Monnier et al., 2000 ; this paper |
| i15 | c.1603-2A>G | Splicing defect | | | 1 | ni | Lowe | Böckenhauer et al., 2008 |
| 16 | c.1621C>T | p.Arg541X | | | 5 | France, India, ni | Lowe | LSMD, 2010 ; this paper |
| 16 | c.1680_1683delCTTC | p.Asp560AspfsX4 | | | 1 | France | Lowe | Satre et al., 1999 |
| 16 | c.1681_1682insGACT | p.Phe561X | | | 1 | Spain | Lowe | This paper |
| 16 | c.1692_1693insCCTT | p.Leu564ProfsX11 | | | 1 | Morocco | Lowe | This paper |
| 16 | c.16494_1695insCCTT | p.Leu565PhefsX11 | | | 4 | ni | Lowe | Addis et al., 2004 |
| 16 | c.1709_1710insG | p.Arg570ArgfsX6 | | | 1 | France | Lowe | Monnier et al., 2000 |
| i16 | 1714-2A>G | Splicing defect | | | 2 | Belgium, ni | Lowe | LSMD, 2010 ; this paper |
| 17 | c.1722-1723insT | p.Glu575X | | | 1 | Germany | Lowe | Röschinger et al., 2000 |
| 17 | c.1723G>T | p.Glu575X | | | 1 | Lebanon | Lowe | Satre et al., 1999 |
| 17 | c.1730T>A | Splicing defect / p.Val577Glu | | | 1 | Italy | Lowe | Addis et al., 2004 |
| 17 | c.1737delT | p.Phe579PhefsX64 | | | 1 | ni | Lowe | LSMD, 2010 |
| 17 | c.1751_1752insG | p.Glu585LysfsX21 | | | 1 | France | Lowe | Monnier et al., 2000 |
| 17 | c.1753_1755delGAG | p.Glu585del | | | 1 | France | Lowe | Monnier et al., 2000 |
| 17 | c.1769delG | P.Ser590ThrfsX53 | | | 1 | ni | Lowe | LSMD, 2010 |
| 17 | c.1773C>A | p.Asn591Lys | | | 1 | Turkey | Lowe | This paper |
| 17 | c.1777delG | p.Gly593AspfsX51 | | | 1 | Spain | Lowe | Satre et al., 1999 |
| 17 | c.1780C>T | p.Gln594X | | | 1 | Arabia | Lowe | This paper |
| 17 | 1822_1834del13nt | p.Ser608_Lys312>SerfsX32 | | | 1 | France | Lowe | Monnier et al., 2000 |
| 17 | 1830C>G | p.Tyr613X | | | 1 | ni | Lowe | LSMD, 2010 |
| i17 | c.1879+5G>A | Splicing defect | 0.03 | - | 1 | France | Lowe | This paper |
| i17 | c.1880-5_1880-21del16 | p.Asn627fsX16 | | | 1 | Italy | Lowe | Addis et al., 2004 |
| 18 | c.1901T>C | p.Leu634Pro | | | 1 | The Netherlands | Lowe | Böckenhauer et al., 2008 |
| 18 | c.1923-1924delT | p.Ser642CysfsX4 | | | 1 | ni | Lowe | Böckenhauer et al., 2008 |
| 18 | c.1925_1926delCT | p.Ser642CysfsX9 | | | 1 | North-America | Lowe | Lin et al., 1997 |
| 18 | c.1927_1928delGT | p.Val643AsnfsX9 | 0.21 | +/- | 1 | France | Lowe | This paper |
| 18 | c.1987C>T | p.Arg663X | 0.04 | - | 5 | North-America Finland, France | Lowe | Lin et al., 1997 ; Monnier et al., 2000 ; LSMD, 2010 |
| 18 | c.2002T>G | p.Phe668Val | | | 1 | France | Lowe | Swan et al., 2010 |
| 18 | c.2037T>G | p.Cys679Trp | | | 1 | Italy | Lowe | McCrea et al., 2008 |
| 18 | c.2060T>C | p.Leu687Pro | | | 1 | ni | Lowe | LSMD, 2010 |
| 18 | c.2071A>T | p.Lys691X | | | 2 | ni | Lowe | Böckenhauer et al., 2008 |
| 18 | c.2071delA | p.Lys691LysfsX13 | | | 1 | France | Lowe | Monnier et al., 2000 |
| 18 | c.2083C>T | p.Arg695X | | | 5 | North-America Kuweit, France | Lowe | Lin et al., 1998 ; Monnier et al., 2000 ; Addis et al., 2004 |
| 18 | c.2086G>T | p.Glu696X | | | 1 | Belgium | Lowe | This paper |
| 18 | c.2092delC | p.Pro698LeufsX66 | | | 1 | Israel | Lowe | This paper |

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|---|---|---|---|---|---|---|---|---|
| 19 | c.2194dupC | p.Leu732ProfsX38 | | | 1 | UK | Lowe | This paper |
| 19 | c.2200delG | p.Val734PhefsX8 | | | 1 | Belgium | Lowe | This paper |
| 19 | c.2203_2204insA | p.Pro735HisfsX35 | | | 1 | North-America | Lowe | Lin et al., 1998 |
| 19 | c.2212G>C | p.Glu737Asp | | | 1 | Italy | Dent-2 | Tosetto et al, 2009 |
| 19 | c.2224_2226delGTA | p.Val742del | | | 1 | Cuba | Lowe | This paper |
| 19 | c.2244C>G | p.Tyr748X | | | 1 | ni | Lowe | LSMD, 2010 |
| i19 | c.2256+1G>A | Splicing defect | | | 1 | Germany | Lowe | Keilhauer et al., 2007 |
| 20 | c.2265delG | p.Leu755LeufsX70 | | | 1 | Belgium | Lowe | Monnier et al., 2000 |
| 20 | c.2269C>T | p.Gln757X | | | 1 | France | Lowe | This paper |
| 20 | c.2269delC | p.Gln757ArgfsX68 | | | 1 | ni | Lowe | LSMD, 2010 |
| 20 | c.2297delA | p.Gln766fsX58 | | | 1 | Italy | Lowe | Addis et al., 2004 |
| 20 | c.2303T>A | p.Ile768Asn | | | 1 | France | Lowe | Monnier et al., 2000 |
| 20 | c.2311-2312insT | p.Cys771LeufsX8 | | | 2 | France, Mali | Lowe | This paper |
| 21 | c.2341+1_del6 | Splicing defect | | | 1 | ni | Lowe | Böckenhauer et al., 2008 |
| 21 | c.2360_2361delTG | p.Val787GlyfsX2 | 0.10-0.16 | - | 4 | North-America, India | Lowe | Lin et al., 1997 ; Lin et al., 1998 ; this paper |
| 21 | c.2389G>C | p.Ala797Pro | 0.39 | ++ | 1 | France | Lowe | Monnier et al., 2000 |
| 21 | c.2396C>T | p.Pro799Leu | 0.09 | ++ | 1 | France | Dent-2 | Swan et al., 2010 |
| 21 | c.2402C>T | p.Pro801Leu | 0.22 | | 1 | France | Lowe | Swan et al., 2010 |
| 21 | c.2415C>G | p.Tyr805X | | | 2 | France | Lowe | Monnier et al., 2000 ; this paper |
| 21 | c.2416G>T | p.Glu806X | | | 1 | ni | Lowe | LSMD, 2010 |
| 21 | c.2428C>T | p.Arg810X | 0.27 | | 5 | UK, India | Lowe | Monnier et al., 2000 ; this paper |
| 21 | c.2433T>A | p.Cys811X | | | 1 | ni | Lowe | LSMD, 2010 |
| 21 | c.2464C>T | p.Arg822X | | | 3 | Germany Lebanon, ni | Lowe | This paper |
| 21 | c.2467C>T | p.Gln823X | 0,05 | - | 1 | North-America | Lowe | Lin et al., 1998 |
| i21 | c.2469+2T>G | Splicing defect | 0.03 | | 1 | French | Lowe | This paper |
| i21 | c.2469+2_6delinsA | Splicing defect | | | 1 | ni | Lowe | LSMD, 2010 |
| 22 | c.2470_2481del12 | p.Val824_Gln27del | | | 1 | North-America | Lowe | Leahey et al., 1993 |
| 22 | c.2515_2516insC | p.Leu839SerfsX12 | | | 1 | ni | Lowe | LSMD, 2010 |
| 22 | c.2530C>T | p.Arg844X | | | 4 | North-America | Lowe | Leahey et al., 1993 |
| 22 | c.2533G>T | p.Glu845X | | | 1 | France | Lowe | Monnier et al., 2000 |
| 22 | c.2550_2551insT | p.Glu851X | 0.07 | + | 1 | North-America | Lowe | Gropman et al., 2000 |
| 22 | c.2553_7 del | p.Glu851GlufsX1 | | | 1 | ni | Lowe | Böckenhauer et al., 2008 |
| 22 | c.2581G>A | Splicing defect, p.Ala861Thr | 0.14 | - | 10 | Japan, Brasil, Italy Belgium, France, India | Lowe | Kawano et al., 1998 ; Monnier et al., 2000 ; Addis et al., 2004 ; Böckenhauer et al., 2008 ; LSMD, 2010 ; this paper |
| i22 | c.2581+1G>C | Splicing defect | | | 1 | Spain | Lowe | This paper |
| i22 | c.2581+4A>G | Splicing defect | | | 1 | ni | Lowe | LSMD, 2010 |
| i22 | c.2582-1G>A | Splicing defect | | | 1 | Spain | Lowe | This paper |
| i22 | c.2582-2A>G | Splicing defect | | | 1 | India | Lowe | Sehti et al., 2008 |

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|---|---|---|---|---|---|---|---|---|
| 23 | c.2591_2595delTCACT | p.Phe864X | | | 1 | France | Lowe | This paper |
| 23 | c.2622delC | p.Asn874AsnfsX34 | 0.29 | | 2 | France, ni | Lowe | Satre et al., 1999 ; LSMD, 2010 |
| 23 | c.2637_2638delGA | p.Gln879_Thr880>HisfsX4 | | | 1 | Grece | Lowe | This paper |
| 23 | c.2642_2643insC | p.Pro881ProfsX2 | | | 1 | ni | Lowe | LSMD, 2010 |
| 23 | c.2672T>G | p.Leu891Arg | | | 1 | Maroco | Lowe | This paper |
| 1-3 | del exons 1-3 | Genomic deletion, frameshift | | | 1 | France | Lowe | Monnier et al., 2000 |
| 1-4 | del exons 1-4 | Genomic deletion, frameshift | | | 1 | France | Lowe | Monnier et al., 2000 |
| 3-4 | del exon 3-4 | Genomic deletion, frameshift | | | 1 | France | Dent-2 | This paper |
| 5-8 | del exons 5-8 | Genomic deletion, frameshift | | | 1 | France | Lowe | Monnier et al., 2000 |
| 6-12 | del exons 6-12 | Genomic deletion, frameshift | | | 2 | North-America | Lowe | LSMD, 2010 |
| 8-12 | del exons 8-12 | Genomic deletion | | | 1 | Belgium | Lowe | Monnier et al., 2000 |
| 14 | del exon 14 | Genomic deletion, frameshift | 0,10 | - | 1 | North-America | Lowe | Lin et al., 1997 |
| 19-23 | del exons 19-23 | Genomic deletion, frameshift | 0,19 | | 1 | Canada | Lowe | This paper |
| 1-23 | del OCRL | Genomic deletion, | | | 2 | Australia, Italy | Lowe | Peverall et al., 2000 ; Addis et al., 2007 |

**Supplementary Table S3 : Reported mutations in the OCRL1 gene**

[a] Numbering was based on the cDNA sequence (#NM_000276) wih +1 corresponding to the A of the ATG initiation codon of translation in the reference sequence.
[b] Expected consequences of the mutations with codon 1 corresponding to the ATG initiation codon of translation.
[c] The enzyme activities were normalized versus the normal activity values indicated by the different authors with 1 being the normal average value measured in controls. When 2 or more patients have been tested for enzyme activity, extreme values are indicated.
[d] The amount of OCRL1 present in patients' fibroblasts was estimated by western blot with ++++ indicating a normal amount and - a complete absence of the protein.
[e] Total number of independent patients reported with the specific mutation.
[f] ni: geographical origin of the patient not indicated